A NEW GENUS, *HESPEROCYPARIS*, FOR THE CYPRESSES OF THE WESTERN HEMISPHERE (CUPRESSACEAE)

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ABSTRACT

Phylogenetic comparisons of three nuclear DNA gene regions (nrDNA(ITS), 4-coumarate: CoA ligase, abscisic acid-insensitive 3) and a chloroplast region (petN-psbM) show that the Western Hemisphere cypresses (*Hesperocyparis*) is a well-supported clade quite separated from the Eastern Hemisphere cypresses (*Cupressus*). Based on these new data and previous data, a new genus, *Hesperocyparis*, is erected for the Western Hemisphere species previously placed in the genus *Cupressus* (*sensu lato*). *Hesperocyparis* is most closely related to the northwestern North American *Callitropsis nootkatensis* and the southeast Asian *Xanthocyparis vietnamensis*. Morphological characters distinguishing *Hesperocyparis* from *C. nootkatensis* and *X. vietnamensis*, and from the Eastern Hemisphere cypresses (*Cupressus*), are presented. *Phytologia 91(1):160-185 (April, 2009)*.

KEY WORDS: *Cupressus*, *Callitropsis*, *Chamaecyparis*, *Hesperocyparis, Xanthocyparis, Juniperus*, nrDNA(ITS), 4-coumarate: CoA Ligase, Abscisic acid-insensitive 3, petN, psbM, sequences, taxonomy.

Attempts to identify and delimit coniferous genera have been "based on limited sets of usually selective characters which were perceived to be informative about evolution and/or phylogeny of the group ... under study *a priori*" (Farjon, 2005). Despite the numerous taxonomic works addressing the Cupressaceae *(sensu lato)*, which were described in detail by Farjon (2005), the modern concept of *Cupressus* has remained largely unchanged for more than a century. However, the discovery of a new conifer species on karst limestone in northern Vietnam (Averyanov et al., 2002; Farjon et al., 2002) has led to both excitement and taxonomic difficulties. Farjon et al. (2002) recognized the taxon as a new species and genus, *Xanthocyparis vietnamensis* Farjon & T. H. Nguyên, based on dimorphic leaves; small ovulate cones with 2 or 3 pairs of opposite decussate cone scales; 2 years for seed cone maturation; flattened, winged seeds; and juvenile, transition, and adult leaves found on the same tree.

Farjon et al. (2002) concluded that *Chamaecyparis nootkatensis* (D. Don) Spach was congeneric with *X. vietnamensis* and included the former species in the new genus (overlooking the earlier generic name *Callitropsis* Oersted) and made the new combination *Xanthocyparis nootkatensis* (D. Don) Farjon & D. K. Harder. *Chamaecyparis nootkatensis* has had a variable taxonomic history, having been classified as *Chamaecyparis, Cupressus, Callitropsis* and *Xanthocyparis* (see Little et al., 2004, and Debreczy et al., 2009 for discussion).

Little et al. (2004), using nrDNA(ITS) internal transcribed spacer (ITS) sequence data, found that *Xanthocyparis vietnamensis* and *X. nootkatensis* form a clade sister to the Western Hemisphere cypresses and that the Eastern Hemisphere cypresses and *Juniperus* constitute distinct clades outside this group. Little et al. (2004) reported that *Chamaecyparis nootkatensis* had been previously described as *Callitropsis nootkatensis* (D. Don) Oersted in 1865. Little renamed *X. vietnamensis* as *Callitropsis vietnamensis* (Farjon and Nguyên) D. P. Little. Though Silba (2005) did not address the molecular phylogeny results, he did assert in response to Little et al. (2004) that the splitting of western and Eastern Hemisphere cypresses was "based on superficial data with inaccurate and incomplete field observations." More recently, Mill and Farjon (2006) made a proposal to conserve

Xanthocyparis against *Callitropsis*. The Nomenclature Committee for Vascular Plants voted 14-4 to recommend that the proposal be adopted at the next International Association of Plant Taxonomists congress in 2012 (Brummitt, 2007). So, the matter currently remains unsettled (see Debreczy et al., 2009, for discussion).

Xiang and Li (2005) reexamined *Xanthocyparis, Chamaecyparis, Cupressus* and *Juniperus* using nrDNA(ITS) sequences. Though the authors concluded that "it seems appropriate" to place *Xanthocyparis vietnamensis* and *X. nootkatensis* in *Cupressus (sensu lato)*, they also noted that "Assuming the ITS tree reflects species relationships, we need a new genus name for the New World species of *Cupressus* if *Xanthocyparis* is recognized." While Xiang and Li (2005) submerged *X. vietnamensis* in *Cupressus*, the combination *Cupressus vietnamensis* was made previously by Silba (2005) and remade later by Rushforth (2007).

Prior to the recent spate of publications described above, taxonomic work on *Cupressus* largely was focused on the specific and infraspecific level with considerable disagreement as to the number of distinct species to recognize in the genus (see Wolf, 1948; Little, 1970; Farjon, 2005). The classical monograph by Camus (1914) treated the known species of *Cupressus* on a worldwide basis and also included the distinct but related *Chamaecyparis* as a subgenus of *Cupressus*. The most thorough morphological treatment of the Western Hemisphere species of *Cupressus* is the revision of Wolf (1948), who only included the New World species in his study on the grounds that the Eurasian and African species were not readily accessible for detailed population-based field studies. He also stated that "none of [the Old World species] appears closely related to our New World species."

Silba (1983), after reportedly raising seedlings of the 25 taxa he delimited, noted consistent differences in cotyledon number and shape in all Western versus Eastern Hemisphere cypresses (cotyledons 3-4 and acute, versus 2 and obtuse), though he did not think these characters, alone, warranted dividing *Cupressus* into subsections. However, Silba (1994, 1998) later arranged *Cupressus* into two subgenera and seven sections, and designated *Cupressus lusitanica* as the type for his new subgenus, *Indoamericana*, which included Western

Hemisphere taxa of *Cupressus* with some additional Asian species. The name of this subgenus, *Indoamericana*, reflects the generally discredited belief, which Silba (2006) continues to support, that *C. lusitanica* (which occurs in the wild in Mexico and central America) actually originated in Goa, India, from where seed was purportedly collected and introduced into Portugal (Farjon 1993). Apart from this problem, Silba's (1994, 1998) treatment would place some species in multiple sections (Little, 2006).

Little (2006) expanded the scope and depth of his previous work (Little, et al., 2004) and analyzed cpDNA (*matK, rbcL*, and *trnL*) plus two nuclear gene regions: nrDNA(ITS), and NEEDLY for all 16 species of the Western Hemisphere and 12 species of the Eastern Hemisphere (*Cupressus, sensu lato*). The portions of his trees relating to *Xanthocyparis*, *Cupressus*, and *Juniperus* are depicted in figure 1.

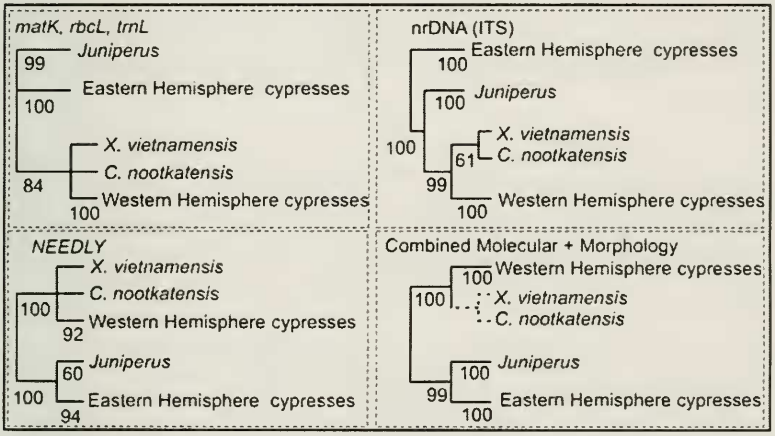

Figure 1. Summary of partial trees extracted from data of Little (2006). Numbers below the branches are strict consensus jackknife frequencies above 50%. Dashed lines in the Combined Molecular + Morphology tree for *C. nootkatensis* and *X. vietnamensis* indicate branches that are collapsed in the strict consensus.

Little (2006) obtained strong support for the Western Hemisphere cypresses as a monophyletic clade in the analyses of each of his molecular data sets. In addition, there was strong support (Fig. 1) that the closest relatives of Western Hemisphere cypresses are *Xanthocyparis vietnamensis* and *Callitropsis nootkatensis*. The Western and Eastern Hemisphere cypresses, and *Juniperus* are each well resolved as distinct clades in each of his molecular analyses, while *X. vietnamensis* and *C. nootkatensis* form an unresolved trichotomy with the Western Hemisphere cypresses in the cpDNA and NEEDLY analyses, and are only moderately well supported (61%) as a 2-species clade in the ITS analysis (Fig. 1). A tree based on morphological data failed to separate eastern from Western Hemisphere cypresses, but a combined analysis of the morphological and molecular data sets did strongly separate these geographic groups (Little, 2006, Fig. 1 above). The results of the molecular and combined analyses also provided strong evidence that *C. lusitanica* is nested well within the Western Hemisphere group, whereas *C. torulosa* is definitely placed within the Eastern Hemisphere lineage contrary to the subgeneric classification of Silba (1994).

In addition to the previously mentioned response to Little et al. (2004) by Mill and Farjon (2006), Farjon (2007) in a letter to a Taxon took issue with Little's 2006 paper because Farjon maintained that the only significant morphological difference between the Western and Eastern Hemisphere cypresses, cotyledon number, did not hold for two Asian species, *C. chengiana* and *torulosa*. Farjon (2007) concluded that there "are no morphological or anatomical differences that justify this generic separation." While acknowledging that "No single characteristic can be used diagnostically," Little (2006) stated that a suite or "series of vegetative characteristics possibly associated with adaption to arid environments (e.g., monomorphic leaves, penultimate and ultimate segments arranged on two planes) unite the New World *Cupressus* species to the exclusion of Old World *Cupressus*, *Juniperus*, and *Callitropsis* [*sensu stricto*]."

Little (2006) decided to include the Western Hemisphere cypresses (*Cupressus*) in *Callitropsis* and published 17 new names from North America. Little (2006) reasoned that giving a new genus name to the Western Hemisphere cypresses (as suggested by Xiang and

Li, 2005) would be "consistent with some but not all resolutions of the polytomy between *Callitropsis* [i.e., *X. vietnamensis* and *Ch. nootkatensis*] and the New World *Cupressus* species." However, only the ITS data (Fig. 1) present *X. vietnamensis* - *C. nootkatensis* as a clade and Little's results did not provide any strong evidence against placing these two taxa in a separate genus (as suggested by Farjon et al., 2002 and Little et al., 2004) or as monotypic genera as suggested by Debreczy et al. (2009).

In an effort to add additional molecular data to the taxonomic questions, we have sequenced two nuclear genes (4-coumarate: CoA ligase, 4CL and abscisic acid-insensitive 3, ABI3, as well as complete nrDNA(ITS) sequences for additional taxa and a cpDNA region, petN-psbM.

The 4-coumarate: CoA Ligase (4CL) gene family is important in phenylpropanoid synthesis leading to lignin, as well as flavonoids, and other pigments as well as phenolic compounds in essential oils such as safrole, eugenol, etc. (Hamberger and Hahlbrock, 2004; Cukovic et al., 2001) Recently, Peng and Wang (2008) utilized 4CL sequences to study *Thuja* species and *Thujopsis dolabrata*. In *Thujopsis dolabrata* they found the 4CL gene to be composed of 4 exons and 3 introns. Intron 2 was reported as 640 bp (EU183423). Aligning the GenBank sequences for *Thuja plicata* (EU183418, EU183417) and *Thujopsis dolabrata* (EU183423) enabled us to design primers to span intron 2, and resulted in 746 - 823 bp of sequence data.

Lazarova, Zeng and Kermode (2001) reported on the occurrence of an abscisic acid-insensitive 3 (ABI3) gene homologue from *Chamaecyparis nootkatensis* (CnABI3). The ABI3 gene is composed of six exons and five introns, with the intron sizes of 105, 113, 110, approx. 1000, and 142 bp. Primers were designed in exon 4 and exon 5 to amplify intron 4 (see Materials and Methods below) and resulted in 1020 - 1108 bp of sequence data.

The cp region trnC-trnD has been used in phylogenetic studies in *Juniperus* (Adams, 2007; Adams et al., 2007). The partial sequence utilized in this study is the petN - psbM region (included in the trnC-trnD region). This region is much easier to amplify and resulted in

approximately 807-854 bp compared to 1400 - 1500 for the full trnC-trnD region.

The purpose of the present study is to bring additional molecular data to bear on the question of the taxonomic status of *Xanthocyparis*, versus the Eastern and Western Hemisphere cypresses.

MATERIALS AND METHODS

Specimens used in this study:

Species	Voucher	Source	GenBank
X. vietnamensis			
nrDNA(ITS)	Little et al. 2004	Vietnam	AY380877
4CL	Rushforth 7745	Vietnam	FJ744493
CnABI3	Rushforth 7745	Vietnam	FJ56803
petN-psbM	Rushforth 7745	Vietnam	FJ46729
C. nootkatensis			
nrDNA(ITS)	Little et al. 2004	AK, USA	AY380858
4CL	*Adams 9086*	WA, USA	FJ744494
CnABI3	*Adams 9086*	WA, USA	FJ56803
petN-psbM	*Adams 9086*	WA, USA	FJ46730
C. atlantica			
nrDNA(ITS)	Little et al. 2004	Morocco	AY988367
4CL	*Adams 8429*	Morocco	FJ744495
CnABI3	*Adams 8429*	Morocco	FJ56805
petN-psbM	*Adams 8429*	Morocco	FJ46731
C. dupreziana,			
nrDNA(ITS)	Little et al. 2004	Algeria ex Hillier Gard.	AY988375
4CL	*Adams 8432*	Algeria ex Hillier Gard.	FJ744496
CnABI3	*Adams 8432*	Algeria ex Hillier Gard.	FJ56806
petN-psbM	*Adams 8432*	Algeria ex Hillier Gard.	FJ46733
C. sempervirens,			
nrDNA(ITS)	*Adams 8434*	Elburz Mts., Iran	FJ705221
4CL	*Adams 8434*	Elburz Mts., Iran	FJ744497
CnABI3	*Adams 8434*	Elburz Mts., Iran	FJ56807
petN-psbM	*Adams 8434*	Elburz Mts., Iran	FJ46732

H. abramsiana			
nrDNA(ITS)	*Adams 9354*	CA, USA	FJ705220
4CL	*Adams 9354*	CA, USA	FJ744498
CnABI3	*Adams 9354*	CA, USA	FJ56808
petN-psbM	*Adams 9354*	CA, USA	FJ46737
H. bakeri			
nrDNA(ITS)	Little et al. 2004	CA, USA	AY988369
4CL	*Adams 9362*	CA, USA	FJ744499
CnABI3	*Adams 9362*	CA, USA	FJ56809
petN-psbM	*Adams 9362*	CA, USA	FJ46739
H. pygmaea			
nrDNA(ITS)	*Adams 9357*	CA, USA	FJ705219
4CL	*Adams 9357*	CA, USA	FJ744500
CnABI3	*Adams 9357*	CA, USA	FJ56810
petN-psbM	*Adams 9357*	CA, USA	FJ46738
J. monticola			
nrDNA(ITS)	*Adams 6876*	HID, MX	FJ705218
4CL	*Adams 6876*	HID, MX	FJ744501
CnABI3	*Adams 6876*	HID, MX	FJ56811
petN-psbM	*Adams 6876*	HID, MX	FJ46736
J. saltillensis			
nrDNA(ITS)	*Adams 6886*	NL, MX	FJ705217
4CL	*Adams 6886*	NL, MX	FJ744502
CnABI3	*Adams 6886*	NL, MX	FJ56812
petN-psbM	*Adams 6886*	NL, MX	FJ46735
J. virginiana			
nrDNA(ITS)	*Adams 6753*	TX, USA	EF608980
4CL	*Adams 6753*	TX, USA	FJ744503
CnABI3	*Adams 6753*	TX, USA	FJ56813
petN-psbM	*Adams 6753*	TX, USA	FJ46734
Thujopsis dolabrata			
nrDNA(ITS)	Peng and Wang	Jiangxi, China	EU183443
4CL	Peng and Wang	Jiangxi, China	EU183423
CnABI3	*Adams 9502*	Japan ex Arn. Arb.	FJ56814
petN-psbM	*Adams 9502*	Japan ex Arn. Arb.	FJ46727

Thuja plicata			
nrDNA(ITS)	*Adams 9277*	Vancouver Isl., BC	AY380852
4CL	Peng and Wang	Kew Bot. G. ex USA?	EU183417
CnABI3	*Adams 10311*	Queen Charlotte Isl., BC	FJ56815
petN-psbM	*Adams 10311*	Queen Charlotte Isl., BC	FJ46728

Specimens only used for size determination of 4CL:
C. arizonica, Adams 9378, Pima Co., AZ; *C. benthamii, Adams 8710*, Pachuca, MX; *C. forbesii, Adams 9370*, San Diego Co., CA; *C. glabra Adams 9389*, Gila Co., AZ; *C. goveniana, Adams 11544*, Monterey Co., CA; *C. guadalupensis, Adams 8417,* Guadalupe Isl., MX, ex Berkeley Bot. Garden; *C. lusitanica, Adams 7071,* cultivated, Bussaco, Portugal; *C. macnabiana, Adams 9359*, Napa Co., CA; *C. macrocarpa, Adams 11459*, Crocker Grove, CA; *C. montana, Adams 9660*, Baja, MX; *C. nevadensis, Adams 9367*, Kern Co., CA; *C. sargentii, Adams 9348*, San Luis Obispo Co., CA; *C. stephensonii, Adams 9376*, San Diego Co., CA. Voucher specimens for Adams collections are deposited at BAYLU. Bartel specimens are held in his personal herbarium.

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit as per manufacturer's instructions.

Amplification and sequencing

ITS (nrDNA), 4CL and trnC-trnD amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E or K (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM $MgCl_2$ according to the buffer used) 1.8 µM each primer.

Gene	Primers	2x buffer	annealing	program	size bp
nrITS	ITSA/ ITSB	K	50°C	(94-50x30)	1077-1105
4CL	4CL49F/4CL814R	G	55°C	(94-55x30)	746-823
CnABI3,	CnABI11F/357R	D	55C	(94-55-x30)	1020-1108
petN	petN5F/psbM111R	E	50°C	(94-50x30)	807-854

Primers (5'-3'):
ITS: ITSA = GGA AGG AGA AGT CGT AAC AAG G;

ITSB = CTT TTC CTC CGC TTA TTG ATA TG.
ITSA and ITSB primers from Blattner (1999).

4CL: 4CL49F AAAGAGCTCATCAAATACAA
4CL814R GAAGAGCTTCCAGCTCAG
4CL primers are from conserved sequences in exon 2 and exon 3 of *Thuja plicata* (EU183418, EU183417) and *Thujopsis dolabrata* (EU1834232) and span intron 2.

CnABI3: CnABI11F AACAATAAGAGCAGGATGTA
CnABI357R CCAGTTTTGGTATCAGAGTA
Addition internal primers utilized:
CnABIint533R CAATATTATCACGCATTTG
CnABIint541R CACAGGAGCAATATTATCAC
CnABIint741R TTACTTGAAACAATCTATTTATGT
CnABI3 primers are from sequences in exon 4 and exon 5 of *Chamaecyparis nootkatensis* (AJ131113) and span intron 4.

petN - psbM:
petN5F: AAC GAA GCG AAA ATC AAT CA
psbM111R: AAA GAG AGG GAT TCG TAT GGA
petN and psbM primers were based on conserved sequences from *Juniperus* species.

The following PCR conditions were used: MJ Research Programmable Thermal Cycler, 30 cycles, 94°C (1 min.), 50°C or 57°C (2 min.), 72°C (2 min.), with a final step of 72°C (5 min.). The PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit. The gel purified DNA band with the appropriate primer was sent to McLab Inc. for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Alignments were made using MAFFT (http://align.bmr.kyushu-u.ac.jp/mafft/) and then manually corrected and then re-analyzed using NJ with 1000 bootstrap replications (http://align.bmr.kyushu-u.ac.jp/mafft/).

We included *Thuja* and *Thujopsis* as outgroup taxa in the analyses following the phylogenies of Gadek et al. (2000) and Little et al. (2004).

RESULTS AND DISCUSSION

The overall sequencing efforts are shown in table 1. The number of informative sites and the percent yield varied from largest in nrDNA(ITS) to smallest in petN-psbM. Clearly, nrDNA(ITS) yielded both the most informative sites and the greatest yield for the effort. The single (or low) copy nuclear genes yielded lots of information, being single genes, are difficult to amplify in amounts for preparative yields. The cp DNA (petN-psbM) is multiple copy and very easy to amplify, but the number and yield of informative sites is somewhat smaller.

Table 1. Summary of sequencing results. # of variable and # of informative sites are within the in-group (excluding *T. dolabrata* and *Th. plicata*). % yield of informative sites (% yield) = 100 x # informative / minimum range observed.

gene	range, bp	# variable	# informative	% yield
nrDNA(ITS)	1077-1105	198	158	14.7%
4CL	746-823	124	79	10.6
CnABI3	1020-1108	137	83	8.1
petN-psbM	807-854	84	57	7.1

Sequencing the nrDNA (ITS region) resulted in 1077 to 1105 bp of sequence data. The ITS tree (Fig. 2) is similar to that of Little (2006, Fig. 1, upper right, above), in that the cypresses from the Eastern and Western Hemispheres are 100% supported as distinct clades. There is some support (75%) for the clade of *C. nootkatensis* - *X. vietnamensis* as reported by Little (2006, 61%, Fig. 1 above). The *C. nootkatensis* - *X. vietnamensis*, clade is allied with the Western Hemisphere cypresses using the ITS data (Fig. 2).

Sequencing of the 4-coumarate: CoA ligase intron 2 (4CL) region resulted 746 - 823 bp of sequence data. Examination of the NJ tree reveals four groups (fig. 3) as found with the nrDNA(ITS) data (Fig. 2). The 4CL tree shows a weak association (34%) between *C.*

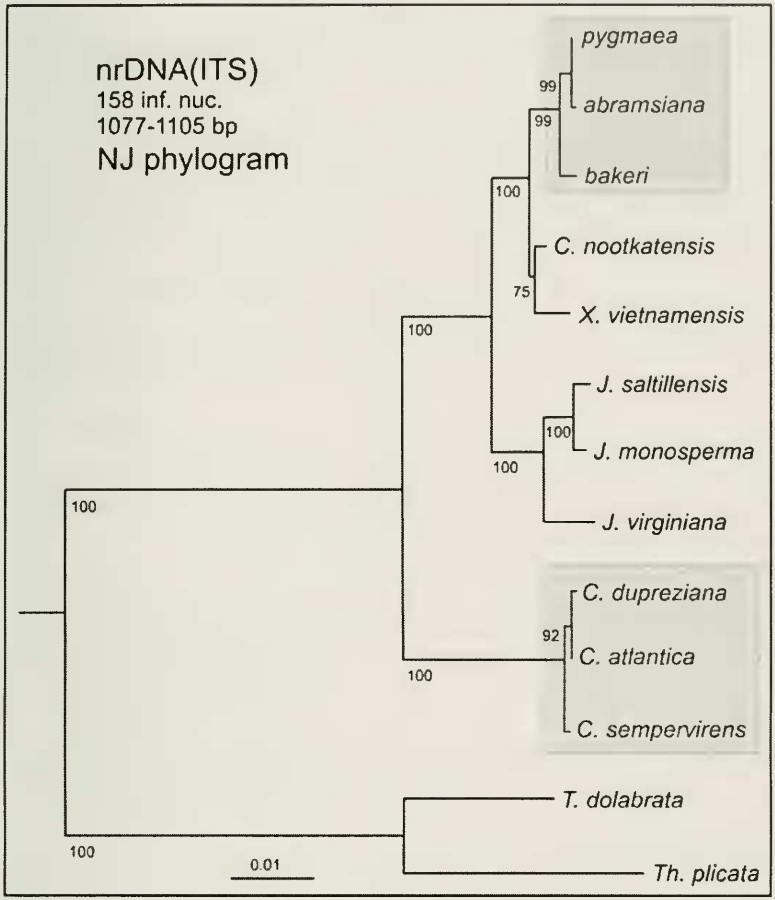

Figure 2. NJ phylogram based on nrDNA(ITS). Numbers below branches are bootstrap probabilities (1000 reps). Eastern Hemisphere cypresses are in the cross-hatched box and Western Hemisphere cypresses are in the shaded box.

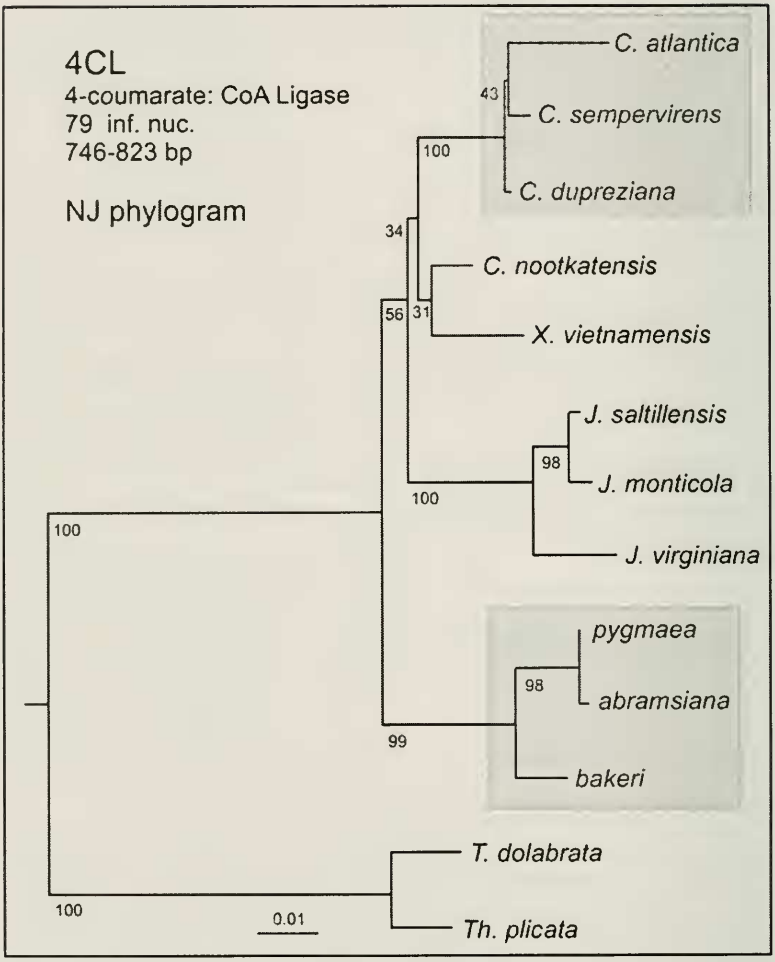

Figure 3. NJ phylogram utilizing sequences from intron 2 of 4-coumarate: CoA ligase (4CL).

nootkatensis-X. vietnamensis and the Eastern Hemisphere cypresses, but again provides substantial support (99%) for the Western Hemisphere cypress clade.

The three Western Hemisphere cypresses (*C. abramsiana, C. bakeri* and *C. pygmaea*) each had a unique 46 bp insert giving them a 4CL length of 817 bp, in contrast, all other taxa that had shorter sequences. A survey of all the other Western Hemisphere cypresses (*C. arizonica, C. benthamii, C. forbesii, C. glabra, C. goveniana, C. guadalupensis, C. lusitanica, C. macnabiana, C. macrocarpa, C. montana, C. nevadensis, C. sargentii, C. stephensonii)* revealed that the length is nearly constant at 817bp, indicating that all these taxa share the 46bp insert.

Sequencing of the CnABI3 intron 4 region revealed several large indels in this data set. The NJ phylogram based on CnABI3 sequence data again shows (Fig. 4) the separate clades of the Eastern and Western Hemisphere cypresses. However, *C. nootkatensis* and *X. vietnamensis* do not form a clade but are well supported as species. The CnABI3 gene sequence supports the contention of Debreczy et al. (2009) that *C. nootkatensis* and *X. vietnamensis* are monotypic genera, since the former forms a strongly supported clade with the Western Hemisphere cypresses (99%).

It is interesting to note that *X. vietnamensis*, *C. atlantica*, *C. dupreziana* and *C. sempervirens* all share a unique 47 bp deletion.

Sequencing petN-psbM of cpDNA resulted lengths ranged from 807 to 854 bp, except for *T. dolabrata* that had only 511 bp. The NJ phylogram (Fig. 5) again shows strong support for separate clades for the Eastern and Western Hemisphere cypresses. Overall, the tree is similar to the cpDNA tree of Little (2006) based on combined sequences from matK, rbcL and trnL (Fig. 1, upper left), but the greater amount of sequence data in Little's tree provides very strong support for the monophyly of the Western Hemisphere cypress lineage (100%), while *C. nootkatensis* and *X. vietnamensis* are not resolved from the Western Hemisphere cypresses in our analysis (Fig. 5). However, again, the Eastern and Western Hemisphere cypresses are in well-supported clades (Fig. 5).

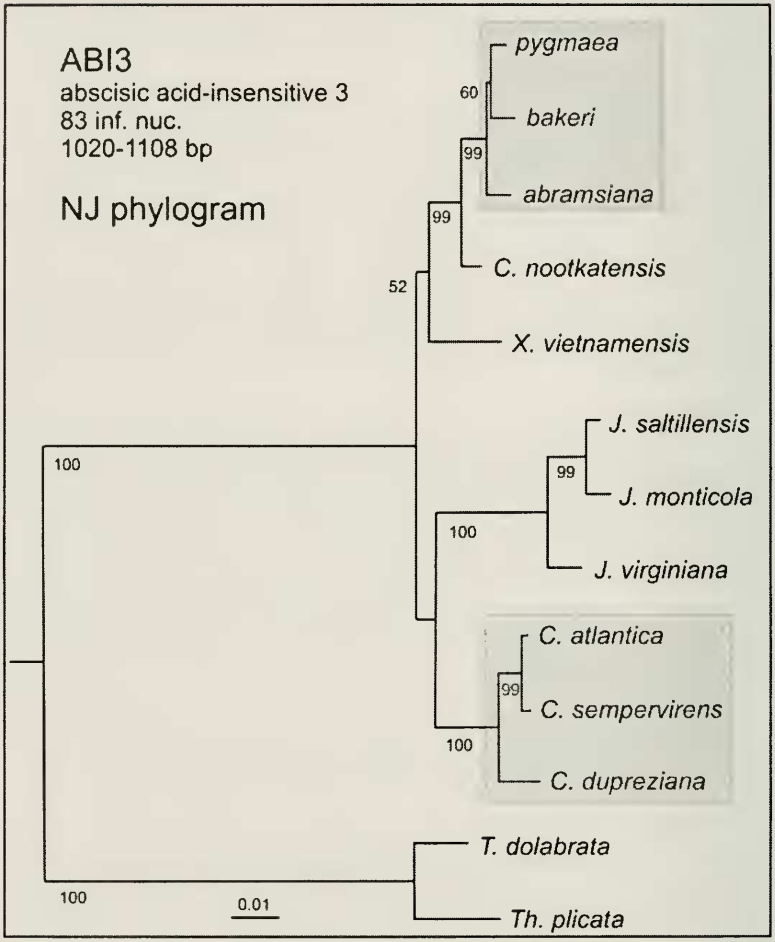

Figure 4. NJ phylogram based on ABI3 intron 4 sequences.

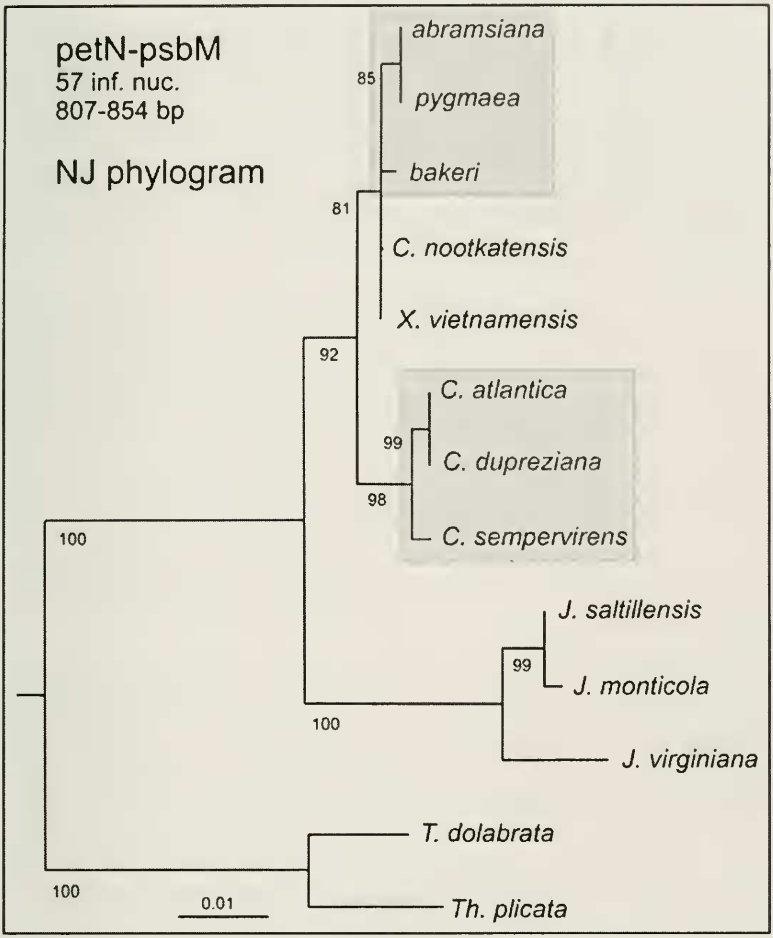

Figure 5. NJ phylogram based on petN - psbM sequences of cp DNA.

An analysis based on combined sequences (nrDNA(ITS), 4CL, CnABI3, petN-psbM) yielding 377 phylogenetically informative nucleotides. The NJ phylogram (Fig. 6) has 100% support for the *T. dolabrata* - *Th. plicata*, Western Hemisphere cypresses, Eastern Hemisphere cypresses, and *Juniperus* clades. It also provides 100% support for the grouping of *C. nootkatensis* and *X. vietnamensis* with the Western Hemisphere cypresses, and is consistent with the proposal by Debreczy et al. (2009) to treat these taxa as monotypic genera.

The separation of *C. nootkatensis* and *X. vietnamensis* is consistent with several morphological characters distinguishing the two: both needle-like juvenile leaves and scale-like adult leaves occur on the mature plant in only the latter, the seed coat has minute warty resin pustules in only the latter, and there is a short but quite distinct resin-filled columella at the center of the mature and open seed cone in only the former (Debreczy et al., 2009). *Callitropsis nootkatensis* and similar extinct forms also have a substantial fossil record dating back to at least 50 MYA in western North America (Edwards, 1983), which may serve to provide a minimum time depth for the split between this group and the Western Hemisphere cypress lineage.

Our results are consistent with those of Little (2006) in providing further support for a distinct lineage of Western Hemisphere cypresses quite separate from the Eastern Hemisphere cypresses and most closely related to *C. nootkatensis* and *X. vietnamensis*. The Western Hemisphere cypresses differ significantly in cone morphology from the latter two species, notably in having cones with many more seeds (typically 5-20 per cone scale and 60-150 per cone versus 2-4 per cone scale and < 15 per cone), woodier and larger peltate cone scales, and cotyledons, with few exceptions, 3-5 in number versus 2. Thus, rather than following Little (2006), who included the Western Hemisphere cypresses plus *C. nootkatensis* and *X. vietnamensis* in an expanded genus *Callitropsis*, we recognize a new genus including only the Western Hemisphere cypress lineage.

The traditional approach of including the Eastern Hemisphere and Western Hemisphere cypress lineages in a genus to the exclusion of

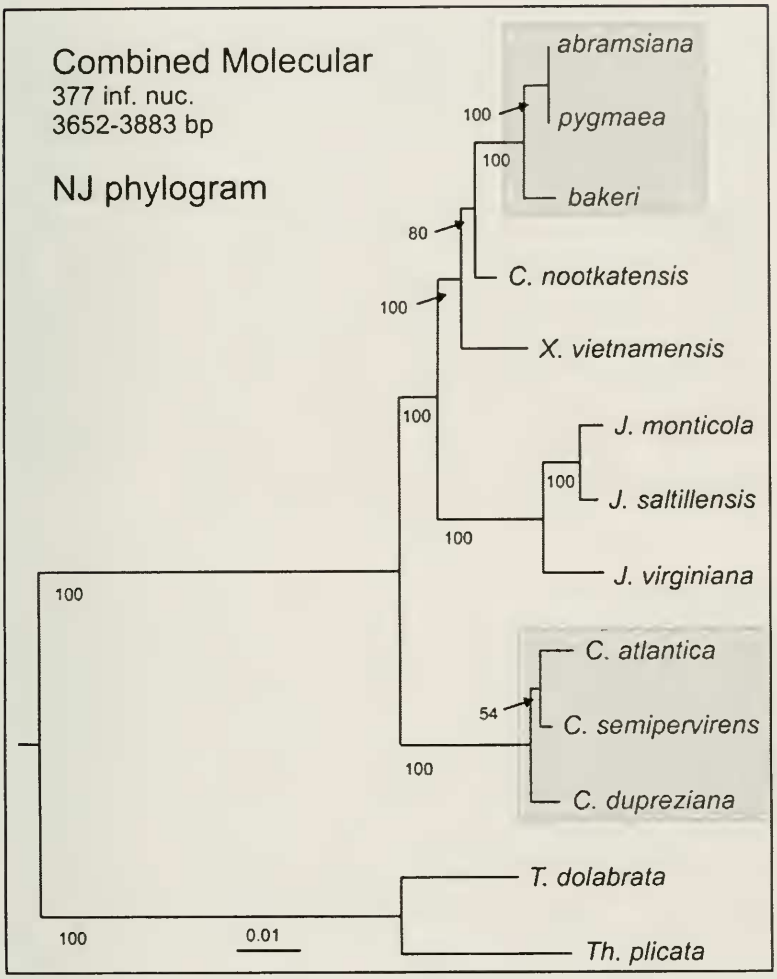

Figure 6. NJ phylogram based on combined sequences (nrDNA(ITS), 4CL, CnABI3, petN-psbM).

Callitropsis, *Xanthocyparis* and *Juniperus* would run contrary to the very likely phylogenetic relationships in the group supported by multiple lines of molecular phylogenetic data, which indicate that *C. nootkatensis* and *X. vietnamensis* rather than the Eastern Hemisphere cypresses are the closest relatives of the Western Hemisphere cypresses (Figs. 1, 6). We believe that the possible alternative approach of including the entire clade of cypresses, junipers, *Xanthocyparis* and *Callitropsis* in a single genus would be unduly disruptive to the nomenclature of horticulturally important taxa, particularly if *Cupressus* is given nomenclatural priority over *Juniperus* (which would require 67 new combinations at the species level; Adams, 2008), and would also tend to obscure rather than elucidate the morphological groupings and major evolutionary lineages in the group. We provisionally recognize 16 species as distinct for purposes of providing new species combinations, following the monographic treatment of Wolf (1948) and the phylogenetic results of Little (2006).

The new genus is cryptic in its macromorphology, being similar to *Cupressus stricto sensu* in its general appearance and cone morphology, but is very distinct in molecular phylogenetic analyses from multiple genes and two genomes. In morphology, it is most evidently distinguished from the majority of species of Cupressus *stricto sensu* in its greater number of cotyledons (3-5), and is distinguished from any taxa of Eastern Hemisphere cypresses that may have parallelisms for this character (*C. torulosa* of Asia has 3-5 cotyledons according to Camus, 1914) by a combination of branchlet characters as described below. *Cupressus torulosa* and all other native Eurasian and African species of the genus are unequivocally placed in the Eastern Hemisphere clade in the molecular phylogenetic analyses of Little (2006). Recognition of new genera as new sources of phylogenetic information emerge to support them as distinct evolutionary units has a long tradition, as witness the segregation of multiple genera of Cupressaceae with similar cone morphology from the classical genus Libocedrus. These segregate genera are now widely recognized (Farjon, 1998, 2005) and are well supported by recent molecular studies (Gadek et al., 2000).

TAXONOMIC TREATMENT

Hesperocyparis Bartel & R. A. Price, **gen. nov.**—
TYPE: *Hesperocyparis macrocarpa* (Hartw. ex Gordon) Bartel.

Differt a *Callitropse* and *Xanthocypare* cotyledonibus 3–5 (vs. 2), squamis strobilis paribus 3–6 (vs. 2–3) peltatis non dense incrassatis (vs. basifixis non dense incrassatis), et seminibus per strobilum generaliter 60–150 (vs. paucioribus quam 15). Differt a *Cupresso* cotyledonibus 3–5 (vs. plerumque 2), testa generaliter glauco (vs. non glauco), ordinibus ultimis duobus segmentis caulinis in fasciculis 3-dimensionalibus, segmentis ultimis caulibus in sectione transversali non complanatis, et foliis monomorphis segmentorum caulinorum ultimorum (vs. ordinibus ultimis duobus segmentis caulinis in asperginibus 2-dimensionalibus aut segmentis caulinis ultimis in sectione transversali complanatis et foliis dimorphis segmentorum caulinorum ultimorum). Plantae Hemisphaerii Occidentalis.

Hesperocyparis differs from *Callitropsis* and *Xanthocyparis* in its cotyledons 3-5 (vs 2), seed cone scales in 3-6 pairs (vs 2-3 pairs), peltate and heavily thickened (vs basifixed and not heavily thickened), and seeds per cone generally 60-150 (vs < 15). *Hesperocyparis* differs from *Cupressus* in its cotyledons 3-5 (vs usually 2), seed coat generally ± glaucous (vs not glaucous), usually ultimate 2 orders of branch segments in 3-dimensional clusters, ultimate branch segments not flattened in cross section, and ultimate branch segments leaves monomorphic (vs usually ultimate 2 orders of branch segments in 2-dimensional sprays, or ultimate branch segments flattened in cross section and ultimate branch segments leaves dimorphic). Plants of the Western Hemisphere.

Shrub or tree to (<1-)4-35(-40) m, multi- to generally single-trunked, monoecious, evergreen. *Bark* on trunk fibrous or leathery and smooth, exfoliating in fibrous strips or irregular-shaped plates, gray to brown to cherry-brown. *Branch segments* (stems and overlapping leaves) terete to quadrangular, ultimate and penultimate branch segments generally in 3-dimensional clusters or rarely in 2-dimensional flattened sprays. *Leaves* of juvenile plants awl- to needle-like,

decussate or in whorls of 3; of adult plants decussate, scale-like, appressed, overlapping, generally monomorphic, minutely denticulate or rarely entire, often with a dorsal resin gland, leaves on vigorously growing shoots more elongate and acute-tipped. *Pollen cones*, terminal on separate ultimate branch segments, sub-spheric to elliptic-ovoid to cylindrical, terete to quadrangular, 2.0-6.5 mm long, 1.3-3.0 mm wide, yellow-green; microsporophylls decussate in 3-10 pairs, 3-6(10) sporangia in an irregular row per microsporophyll. *Seed cones* 10-50 mm long, more or less woody, nearly spheric to widely cylindric, maturing in the second year, generally remaining closed at maturity and opening after many years or in response to fire, abscising after opening or after many additional years; scales decussate in (2-)3-6 pairs, thickened, peltate, abutting, shield- or wedge-shaped, boss generally >1 mm (especially prior to maturity), pointed, base level with or rising from edge. *Seeds* many per scale (generally 5-20) per cone, flattened, ovate to lenticular, irregularly faceted due to close packing; seed wings, 2, membranous, narrow, seed body light tan to red brown to brown to dark brown to black, generally glaucous, generally warty with minute resin pustules in the seed coat; cotyledons (2-)3-5(-6), linear, slightly ridged, bluntly pointed at apex. Chromosome number, 2n = 22(23,24).

Hesperocyparis abramsiana (C. B. Wolf) Bartel, **comb. nov.** *Cupressus abramsiana* C. B. Wolf, Aliso 1: 215. 1948. *Cupressus goveniana* Gordon var. *abramsiana* (C. B. Wolf) Little, Phytologia 20: 435. 1970. *Cupressus goveniana* Gordon subsp. *abramsiana* (C. B. Wolf) A. E. Murray, Kalmia 12: 19. 1982. *Callitropsis abramsiana* (C. B. Wolf) D. P. Little, Syst. Bot. 31: 473. 2006.

Hesperocyparis arizonica (Greene) Bartel, **comb. nov.** *Cupressus arizonica* Greene, Bull. Torrey Bot. Club 9: 64. 1882. *Cupressus benthamii* Endl. var. *arizonica* (Greene) Mast., J. Linn. Soc. Bot. 31: 340. 1896. *Callitropsis arizonica* (Greene) D. P. Little, Syst. Bot. 31: 473. 2006.

Hesperocyparis bakeri (Jeps.) Bartel, **comb. nov.** *Cupressus bakeri* Jeps., Fl. Calif. 1: 61. 1909. *Cupressus macnabiana* A. Murray bis var. *bakeri* (Jeps.) Jeps., Man. Fl. Pl. Calif. 58.

1923. *Callitropsis bakeri* (Jeps.) D. P. Little, Syst. Bot. 31: 473. 2006.

Hesperocyparis benthamii (Endl.) Bartel, **comb. nov.** *Cupressus benthamii* Endl., Syn. Conif. 59. 1847. *Cupressus lusitanica* Mill. var. *benthamii* (Endl.) Carrière, Traité Gén. Conif., ed. 2, 155. 1867. *Cupressus lusitanica* Mill. subsp. *benthamii* (Endl.) Franco, Agros (Lisbon) 28: 24. 1945. *Callitropsis benthamii* (Endl.) D. P. Little, Syst. Bot. 31: 473. 2006.

Hesperocyparis forbesii (Jeps.) Bartel, **comb. nov.** *Cupressus forbesii* Jeps., Madroño 1: 75. 1922. *Cupressus guadalupensis* S. Watson var. *forbesii* (Jeps.) Little, Phytologia 20: 435. 1970. *Cupressus guadalupensis* S. Watson subsp. *forbesii* (Jeps.) R. M. Beauch., Aliso 9: 191. 1978. *Callitropsis forbesii* (Jeps.) D. P. Little, Syst. Bot. 31: 473. 2006.

Hesperocyparis glabra (Sudw.) Bartel, **comb. nov.** *Cupressus glabra* Sudw., Amer. Forest. 16: 88. 1910. *Cupressus arizonica* Greene var. *glabra* (Sudw.) Little, Madroño 18: 162. 1966. *Cupressus arizonica* Greene subsp. *glabra* (Sudw.) A. E. Murray, Kalmia 12: 19. 1982. *Callitropsis glabra* (Sudw.) D. P. Little, Syst. Bot. 31: 473. 2006.

Hesperocyparis goveniana (Gordon) Bartel, **comb. nov.** *Cupressus goveniana* Gordon, J. Hort. Soc. London 4: 295. 1849. *Callitropsis goveniana* (Gordon) D. P. Little, Syst. Bot. 31: 473. 2006.

Hesperocyparis guadalupensis (S. Watson) Bartel, **comb. nov.** *Cupressus guadalupensis* S. Watson, Proc. Amer. Acad. Arts 14: 300. 1879. *Cupressus macrocarpa* Hartw. var. *guadalupensis* (S. Watson) Mast., Gard. Chron., Ser. 3 18: 62. 1895. *Callitropsis guadalupensis* (S. Watson) D. P. Little, Syst. Bot. 31: 473. 2006.

Hesperocyparis lusitanica (Mill.) Bartel, **comb. nov.** *Cupressus lusitanica* Mill., Gard. Dict., ed. 8. *Cupressus* no. 3. 1768.

Callitropsis lusitanica (Mill.) D. P. Little, Syst. Bot. 31: 474. 2006.

Hesperocyparis macnabiana (A. Murray bis) Bartel, **comb. nov.** *Cupressus macnabiana* A. Murray bis, Edinburgh New Philos. J. ser. 2, 1: 293. 1855. *Callitropsis macnabiana* (A. Murray bis) D. P. Little, Syst. Bot. 31: 474. 2006.

Hesperocyparis macrocarpa (Hartw. ex Gordon) Bartel, **comb. nov.** *Cupressus macrocarpa* Hartw. ex Gordon, J. Hort. Soc. London 2: 187. 1847. *Callitropsis macrocarpa* (Hartw.) D. P. Little, Syst. Bot. 31: 474. 2006.

Hesperocyparis montana (Wiggins) Bartel, **comb. nov.** *Cupressus montana* Wiggins, Contr. Dudley Herb. 1: 161. 1933. *Cupressus arizonica* Greene var. *montana* (Wiggins) Little, Madroño 18: 163. 1966. *Cupressus arizonica* Greene subsp. *montana* (Wiggins) A. E. Murray, Kalmia 15: 11. 1985. *Callitropsis montana* (Wiggins) D. P. Little, Syst. Bot. 31: 474. 2006.

Hesperocyparis nevadensis (Abrams) Bartel, **comb. nov.** *Cupressus nevadensis* Abrams, Torreya 19: 92. 1919. *Cupressus macnabiana* A. Murray bis var. *nevadensis* Abrams (Abrams), Ill. Fl. Pacific States 1: 73. 1923. *Cupressus arizonica* Greene var. *nevadensis* (Abrams) Little, Madroño 18: 164. 1966. *Cupressus arizonica* Greene subsp. *nevadensis* (Abrams) A. E. Murray, Kalmia 12: 19. 1982. *Callitropsis nevadensis* (Abrams) D. P. Little, Syst. Bot. 31: 474. 2006.

Hesperocyparis pygmaea (Lemmon) Bartel, **comb. nov.** *Cupressus goveniana* Gordon var. "*pigmaea*" Lemmon, Handb. W. Amer. Cone-bearers. ed. 3, 77. 1895. *Cupressus pygmaea* (Lemmon) Sarg., Bot. Gaz. (Crawfordsville) 31: 239. 1901. *Cupressus goveniana* Gordon subsp. *pygmaea* (Lemmon) A. Camus, [Les Cyprès] Encycl. Econ. Sylvicult. 2: 50. 1914. *Callitropsis pigmaea* (Lemmon) D. P. Little, Syst. Bot. 31: 474. 2006.

Hesperocyparis sargentii (Jeps.) Bartel, **comb. nov.** *Cupressus sargentii* Jeps., Fl. Calif. 1: 61. 1909. *Cupressus goveniana* Gordon var. *sargentii* (Jeps.) A. Henry, in Elwes & A. Henry, Trees Great Britain 1173. 1910. *Callitropsis sargentii* (Jeps.) D. P. Little, Syst. Bot. 31: 474. 2006.

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